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Part II

Department of Health, Education, and Welfare

National Institutes of Health

Recombinant DNA Research; Announcement of Meeting of Advisory Committee and Notice of Proposed Actions Under Guidelines

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Pub. L. 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee at the National Institutes of Health, Conference Room 6, Building 31C, Bethesda, Maryland 20205, on June 5, 1980, from 9:00 a.m. to recess at approximately 6:00 p.m., and on June 6, 1980, from 8:30 a.m. to 5:00 p.m. This meeting will be open to the public on June 5 from 9:00 a.m. to recess at approximately 6:00 p.m., and on June 6 from 8:30 a.m. to approximately 3:00 p.m. to discuss:

Amendment of Guidelines
Containment practices appropriate for large-scale production
Voluntary compliance with Guidelines
Institutional Biosafety Committees
Exceptions to prohibitions
Exemptions for organisms that exchange genetic information
Procedures for lowering containment for characterized clones and purified DNA
Review of large-scale proposals
E. coli K–12 host-vector systems
Host-vector systems other than *E. coli* K–12
NIH risk-assessment plan
Review of protocols for required containment levels
Other matters requiring necessary action by the Committee.

Attendance by the public will be limited to space available.

In accordance with provisions set forth in section 552b(c)(4), Title 5, U.S. Code and section 10(d) of Pub. L. 92–463, the meeting will be closed to the public for approximately two hours for the review, discussion and evaluation of proposal(s) from a commercial concern(s) for scale-up of recombinant DNA experiments. It is anticipated that this will occur on June 6, from approximately 3:00 p.m. until adjournment. The proposal(s) and the discussion could reveal confidential trade secrets or commercial property such as patentable material.

Dr. William J. Gartland, Jr., Executive Secretary, Recombinant DNA Advisory Committee, National Institutes of Health, Building 31, Room 4A52, telephone 301–496–6051, will provide materials to be discussed at the meeting, rosters of committee members and substantive program information. A summary of the meeting will be available at a later date.

Dated: April 22, 1980.

Suzanne L. Fremeau,

Committee Management Officer, National Institutes of Health.

[FR Doc. 80–13046 Filed 4–29–80; 8:45 am]

BILLING CODE 4110–08–M

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHEW.

ACTION: Notice of proposed actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth proposals for actions to be taken under the 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules [Federal Register of January 29, 1980 (45 FR 6724)]. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its June 5–6, 1980, meeting, the Director of the National Institutes of Health will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by May 30, 1980.

ADDRESS: Written comments and recommendations should be submitted to the Director, office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following changes and amendments under the Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724), as well as actions under these Guidelines.

1. *Proposal to Amend Section III–O of the Guidelines to Permit Use of Ff Phages.* The National Institutes of Health (NIH) promulgated revised Guidelines in January 1980 (45 FR 6724). The revised Guidelines include a new Section, III–O, "Classification of Experiments Using the *E. coli* K–12 Host-Vector Systems," which, in part, reads as follows:

"* * * experiments using *E. coli* K–12 shall use P1 physical containment and * * * an EK1 host-vector system [i.e. (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid bacteriophages or nonconjugative plasmids shall be used as vectors].* * *"

Section III–O, which was promulgated on the recommendation of the RAC, did not include reference to use of Ff bacteriophages (filamentous single-strand male-specific bacteriophages such as M13 and fd). Consequently, when the revised Guidelines were promulgated in January 1980, investigators were informed that Ff bacteriophages may continue to be used as a component of EK1 systems at the containment levels specified by the 1978 Guidelines. (A complete discussion of this issue appears in the Decision Document that accompanied the November 30, 1979 proposed revised Guidelines (44 FR 69244–69245) and the Decision Document that accompanied the final revised Guiidelines (45 FR 6721)).

In December 1979, the RAC was asked to consider how *E. coli* K–12 host-vector systems employing Ff phages should be treated under the 1980 Guidelines. In response to this NIH request, the Host-Phage Subcommittee drafted a recommendation which was presented to the full RAC at the March 6–7, 1980 meeting.

The RAC agreed that the following proposed amendment to Section III–O should be published in the **Federal Register** for a thirty-day comment period (amended text in italics):

"* * * experiments using *E. coli* K–12 shall use P1 physical containment and, * * * an EK1 host-vector system [i.e. (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid *or Ff* bacteriophages or non-conjugative plasmids shall be used as vectors].* * *"

Background documentation is available from ORDA.

2. *Proposed Changes to Allow Recombinant DNA Experiments with Class 3 Organisms and Certain Plant Pathogens.* The National Institute of Allergy and Infectious Diseases (NIAID) requested that the NIH consider whether, and under what conditions, recombinant DNA technology might be used to study CDC Class 3 etiological agents. Experiments employing these agents are currently prohibited by Section I–D–1 of the Guidelines which states as not to be initiated:

"I–D–1. Formation of recombinant DNMAs derived from the pathogenic organisms classified [1] as Class 3, 4 or 5]2] or the cells

known [2A] to be infected with such agents, regardless of the host-vector system used."

This question was presented to the RAC at its December 6–7, 1979 meeting and a working group was appointed to study the issue. A draft proposal of a revision of the Guidelines to remove Class 3 agents from Section I–D–1 of the Guidelines was presented to the RAC at its March 6–7, 1980 meeting.

Prior to the March 6–7, 1980 meeting, Dr. Clarence Kado of the University of California, Davis, requested the RAC to consider the elimination of Section I–D–3 of the Guidelines, which states as not to be initiated:

"I–D–3. Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange. [2A]"

This issue was also discussed by the RAC at the March 6–7, 1980 meeting. During the discussion, it was suggested that the mechanism used to remove CDC Class 3 agents from Section I–D–1 of the Guidelines might be appropriate for removing recombinant DNA experiments involving certain plant pathogens from Section I–D–3. Accordingly the proposals have been combined.

The purpose of the proposed changes is to remove recombinant DNA experiments with CDC Class 3 organisms and certain recombinant DNA experiments with plant pathogens from the prohibited classes of experiments. The proposal would allow experiments with CDC Class 3 organisms to be conducted at P3 containment in *E. coli* K–12 EK1 host-vector systems. The containment levels for other recombinant DNA experiments would be assigned by the director, NIH after review by the RAC.

It is felt that reducing administrative procedures will increase flexibility and encourage the use of these techniques in elucidating the pathogenic process. P3 containment, which is considered adequate for work with the pathogenic organisms themselves, should be adequate, or more than adequate, for work with *E. coli* K–12 containing recombinant DNA from these organisms.

Changes are proposed in the Guidelines as follows:

A. Section I–D–1 is to be amended to read:

"I–D–1. formation of recombinant DNAs derived from pathogenic organisms classified [1] as Class 4 or 5 or from cells known [2A] to be infected with such agents, regardless of the host-vector system used."

B. Section I–D–3 is to be deleted.

C. Section I–E is to be amended to read:

"I–E. *Exemptions.* It must be emphasized that the following exemptions [4] are not meant to apply to experiments described in Sections I–D–1 to I–D–5 as being prohibited. In addition, any recombinant DNA molecules involving DNA from Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5."

D. Section III. After the last sentence, "The use of higher levels of biological containment * * * for the purposes of the experiment," the following new paragraph is to be added:

"Experiments involving recombinant DNA from Class 3 organisms (1) or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen will be determined by NIH (See Section IV–E–1–b–2–e)."

E. Section III–O. After the third paragraph, "Some experiments * * * are exempt from the Guidelines (see Section I–E)," the following new paragraph is to be added:

"Experiments using *E. coli* K–12 EK1 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See Section IV–3–1–b–2–e)."

F. A new Section IV–E–1–b–2–e is to be added as follows:

"IV–E–1–b–2–e. Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and for experiments which increase the host-range and virulence of plant pathogens."

G. Section V, footnote 2, is to be amended to read:

"2. For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of recombinant DNA Activities."

H. Section V, footnote 36. The second sentence is to be amended to read:

"(As noted in the Prohibition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)"

3. *Proposal to Amend Portions of Section III–A–2–a, Viruses of Eukaryotes.* In the 1980 Guidelines a level of biological containment requiring the use of an "HV1 host and a vector certified for use in an HV2 system" has been substituted for "an EK1 host and a vector certified for use in an EK2 system." This level of biological containment is abbreviated as "HV1CV" in Table III and Footnote 40 of the 1980 Guidelines. At the December 6–7, 1979 meeting of the RAC, a working group was appointed to review the appropriateness of this level of biological containment. The working group proposed that HV2 biological containment be required for these experiments. Accordingly, a proposal to amend portions of Section III–A–2–a, *Viruses of Eukaryotes,* was published in the **Federal Register** of January 31, 1980 [45 FR 7182].

The RAC discussed this proposal at the March 6–7, 1980 meeting. During the discussion, the RAC agreed that the "CV" nomenclature was developed for EK host-vector systems. However the RAC felt that requiring the use of HV2 systems instead of HV1CV imposed an unnecessarily stringent level of containment.

The RAC voted to publish for comment in the **Federal Register** a proposal to substitute HV1 for HV1CV in all places in the Guidelines. The proposal is as follows: Substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system" in Section III–A–2–a–(1)–(a)–(*3*)–(*b*) and substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1," in Sections III–A–2–a–(1)–(a)–(*2*)–(*c*), III–A–2–a–(1)–(b)–(*1*)–(*b*), III–A–2–a–(1)–(b)–(*2*)–(*b*), III–A–2–a–(2)–(a)–(*1*)–(*b*), III–A–2–a–(2)–(a)–(*2*)–(*b*), and III–A–2–a–(2)–(c)–(*2*)–(*b*). In Table III, whenever any one of the following three terms appears, ("HV1CV [40]"; HV1CV[40] or P3 + HV1"; or "HV1CV[40] or P3 + HV1[38]") it would be changed to "HV1."

4. *Proposal To Include Saccharomyces cerevisiae Host-Vector Systems under Section III–O of the Guidelines.* Dr. Jane Setlow of the Brookhaven National Laboratory, proposed at the March 6–7, 1980 meeting, that the RAC consider classifying under Section III–O of the Guidelines experiments involving laboratory strains of *Saccharomyces cerevisiae* as host-vector systems. Dr. Setlow advanced the following arguments to support this proposal: (1) *S. cerevisiae* is nonpathogenic; (2) it does not implant in the intestine; (3) the dilute conditions in which it is found in nature are extremely unfavorable for mating; and (4) it does not efficiently compete with wild type strains of *S. cerevisiae.* She noted in addition that *S. cerevisiae* does not have a biological association with man. Material supporting this proposal has been provided to ORDA by Dr. Setlow and is available upon request. The RAC discussed this question at the March 6–

7, 1980 meeting, and agreed that the proposal should be published in the Federal Register for comment.

The proposal consists of two parts as follows:

A. The following language would be substituted for Section III–O:

III–O *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems.

These are the systems for which we have the most experience and knowledge.

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are prohibited (see Section I–D).

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).

Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, and HV1 host-vector system [i.e. for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or non-conjugative plasmids [49] shall be used as vectors. For experiments in *S. cerevisiae*, laboratory strains shall be used.] For these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their intiation, investigators must submit to their Instutitional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to intiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 or *S. cerevisiae* efficiently express as a protein product the information carried in any gene derive from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.

Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).

For those not on the Appendix A list but which exchange genetic information (35) with *E. coli*, experiments may be performed with any *E. coli* K–12 vector (e.g. conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages.

B. The language "*HV1—Unmodified laboratory strains of *Saccharomyces cerevisiae*" would be deleted from Appendix D.

5. *Request for Permission to Transform Sacchromycopsis lipolytica with E. coli/S. cerevisiae Hybrid Plasmids.* Dr. David M. Ogrydziak of the University of California, Davis, requests permission to attemt to transform the yeast *Sacchromycopsis lipolytica* with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and to use the hybrid plasmids for shotgunning *S. lipolytica* DNA in *E. coli* and returning the DNA to *S. lipolytica.*

6. *Proposal to Amend Section III–B–3 of the Guidelines.* Dr. Allan Campbell of Stanford University has proposed that the RAC consider amending Section III–B–3 of the Guidelines, which currently reads as follows:

III–B–3. Non-HV1 systems. Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment. (2A)

Dr. Campbell writes that the P3 containment level was assigned because of apprehensions that the category might contain some experiments for which that level was appropriate rather than that all experiments in this category were perceived as requiring P3 containment. His proposed amendments are "intended to assert RAC's intention to review individual proposals in an open-minded manner with respect to realistic evaluation of possible hazard, and to discourage the inference that the P3 level, as it applies to a specific experiment, has been set on the basis of information about the organisms involved."

Dr Campbell proposes changes in the Guidelines as follows:

A. Section III–B–3, paragraph 2, would be amended to read:

"In those cases when genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment [2A]. Lower levels of physical containment may be assigned by NIH for specific donor-recipient combinations. (See Section IV–E–1–b–2–f)."

B. A new section IV–E–1–b–2–(f) would be added to the Guidelines as follows:

"IV–E–1–b–2–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes. (See Section III–B–3)."

An alternate amendment of the second paragraph of Section III–B–3 of the Guidelines has been proposed by Dr. Irving Johnson of Lilly Research Laboratories, as follows (amended language in italics):

"In those cases where genetic exchange has not been demonstrated between two bacterial species A and B neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under *physical containment and conditions specified by the local IBC based on its consideration of the biological properties of the organisms.*"

7. *Request to Delete Language Requiring the use of Cauliflower Mosaic Virus (CaMV) mutants lacking the Aphid Transmission Factor from Section III–C–3. Request for Permission to Transfer DNA fragments from Aphid Transmissible to Aphid Nontransmissible CaMV.* Dr. Robert J. Shepherd of the University of California at Davis has requested that the following paragraph be deleted from section III–C–3 of the Guidelines:

"The CaMV strain used as a cloning vector shall be a mutant that lacks the aphid transmission factor."

In addition, Dr. Shepherd requests that he be permitted to transfer DNA segments from aphid transmissible to non-aphid transmissible strains of cauliflower mosaic virus in order to study the factors determining aphid transmissibility. This experiment could be considered as being prohibited under Section I–D–3 of the Guidelines. Dr. Shepherd suggests that it is inconsistent to restrict studies with cauliflower mosaic virus to non-insect transmissible strains when no such restriction is imposed on studies with bean golden mosaic and related viruses, the only other known group of DNA plant viruses. Dr. Shepherd suggests that under the Pl containment conditions specified in the Guidelines, cauliflower mosaic virus is easily confined to a growth chamber or greenhouse. In addition, the virus has a very restricted host range and is neither seed nor pollen transmitted, providing even further containment.

8. *Proposal to Amend Section III–C–4 of the Guidelines.* Dr. Milton Zaitlin of Cornell University proposed that Section III–C–4 of the Guidelines be amended to redefine a Pl greenhouse or growth chamber when certain infectious agents other than viruses are used as vectors. He proposes that the following

language should be added at the end of III–C–4:

"and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules."

Dr. Zaitlin notes that a Pl greenhouse or growth chamber is described in Section III–C–3, Plant Viral Host-Vector Systems, in which there is a recommendation for positive air pressure in order to exclude insects which might transmit viruses. However, in the conditions considered in Section III–C–4, i.e. Plant Host-Vector Systems other than Viruses, the propagules themselves (spores, mycelia, etc.) should be contained, and a negative air pressure would be more appropriate.

9. *Cloning of Foot and Mouth Disease Virus—Stage II.* At the December 6–7, 1979 meeting, the RAC considered a proposal submitted by investigators at the Plum Island Animal Disease Center to permit the cloning of foot and and mouth disease virus (FMDV) in an *E. coli* K–12 host-vector system. Since FMDV virus is classified as a Class 5 agent, an exception to a prohibition of the Guidelines (I–D–1) was requested for cloning segments of the virus. Stage I of the proposal involves the cloning of double-stranded cDNA from RNA isolated from the virus. Subgenomic fragments of the viral genome are to be propagated in an *E. coli* K–12 host employing plasmid pBR322 as a vector. Clones containing FMDV sequences of interest would be analyzed and collected. The Director, NIH on recommendation of the RAC, approved on January 17, 1980 the formation of recombinants between fragments of FMDV and plasmid pBR322 as outlined in Stage I of the proposal. This stage is being carried out at the Plum Island Animal Disease Center.

The second stage of the scientific plan is proposed to be carried out in a joint effort at the laboratories of Genentech, Inc., South San Francisco, California. After clones containing viral sequences of interest have been assayed for lack of infectivity, they would be transferred to Genentech, Inc. for detailed molecular characterization. An attempt would be made to modify clones to produce polypeptides of interests, specifically the VP_3 polypeptide.

The RAC at its December 6–7, 1979 meeting recommended that a Working Group of the RAC be formed to report back to the full RAC after examination of the testing data on the infectivity of the clones produced at Plum Island under Stage I, prior to their removal from Plum Island. The Director, NIH accepted this recommendation on January 17, 1980. As Stage I of the project is nearing completion, the investigators now propose to submit testing data on the infectivity of the clones to the RAC Working Group and the RAC at its June 5–6 meeting in order to seek approval for further stages of the project. The RAC will consider whether they recommend that the clones may be removed from Plum Island, and what they judge to be the appropriate containment level for work with the clones at Genentech, Inc.

10. *Request for an Exception to a Prohibition: Field Testing of Corn Genes Put into Corn (Zea mays) with an E. coli or S. cerevisiae Vector.* Dr. Ronald Davis of Stanford University Medical Center has requested permission from the RAC to field test corn plants (Zea mays) which have been transformed by corn DNA or modified corn sequences cloned in *Escherichia coli* or *Saccharomyces cerevisiae* host-vector systems. Approval of this request requires an exception to Prohibition I–D–4, which prohibits deliberate release into the environment of any organism containing recombinant DNA.

11. *Request for Certification of a Bacillus subtilis Strain as the Host Component of an HV2 Host-Vector System.* Dr. William F. Burke, Jr. of Arizona State University requests the RAC to certify *Bacillus subtilis* strain ASB298 as the host component of an HV2 host-vector system. Dr. Burke has supplied ORDA with a report outlining the construction and characteristics of this strain.

12. *Proposed Revision of Section III–C–1–e.* A notice appeared in the Federal Register of January 31, 1980 concerning proposed revision of Section III–C–1–e, and its subsections. It was recommended that Sections III–C–1–e, III–C–1–e–(1), III–C–1–e–(1)–(a), and III–C–1–e(1)–(b) of the Guidelines be changed and that a new Section III–C–1–e–(1)–(c) be added. Section III–C–1–e–(2) would remain unchanged. The RAC, at its March 6–7, 1980 meeting, recommended adoption of Sections III–C–1–e, III–C–1–e–(1), and III–C–1–e–(1)–(a) as published in the Federal Register of January 31, 1980, with certain modifications in Section III–C–1–e–(1)–(a).

The Director, NIH, accepted this recommendation and promulgated the following sections in the Federal Register of April 11, 1980:

"III–C–1–e. All Viral Vectors.

III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:

III–C–1–e–(1)–(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus [all viruses from a single Family (36) being considered identical (50)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome."

At its March 6–7, 1980 meeting, the RAC voted to defer consideration until the June 5–6, 1980 meeting of the new Sections III–C–1–e–(1)–(b) and III–C–1–e–(1)–(c) as proposed in the Federal Register of January 31, 1980, and requested that a working group develop additional information. An *ad hoc* working group will meet on May 13, 1980 during the meeting of the American Society for Microbiology. The report of the working group will be considered at the June 5–6, 1980 RAC meeting. Accordingly, the proposed revised Sections III–C–1–e–(1)–(b) and III–C–1–e–(1)–(c) are again published for public comment prior to consideration at the June 5–6, 1980 meeting:

"III–C–1–e–(1)–(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the helper virus are not able to grow in human cells.

III–C–1–e–(1)–(c). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P3 containment if wild type strains of the helper virus are able to grow in human cells."

13. *Lowering of Containment Levels for Characterized Clones or Purified DNAs by ICBs.* The December 1978 NIH Guidelines specified in Sections III–A–3–a, III–A–3–b, and IV–D–3–b, conditions under which the local Institutional Biosafety Committee could, without NIH prior approval, give approval for a single step reduction in physical or biological containment. When the Guidelines were revised in January 1980 and Section III–A was changed from covering experiments with the *E. coli* K–12 host-vector system to covering experiments with certain HV1 and HV2 systems, the ability of the IBC to grant such single step reductions without NIH approval was withdrawn. (This is discussed on page 69246 of the November 30, 1979 Federal Register). The RAC at its March 6–7, 1980 meeting voted 9 to 1 with 3 abstentions that there be published in the Federal Register for comment, and brought back to the RAC at its June 5–6, 1980 meeting, proposed revisions of the Guidelines to permit a single step reduction in physical or biological containment for characterized clones or purified DNA, to be approved by the local IBC, without prior NIH

approval. Accordingly the following changes in the Guidelines are proposed:

A. Section III–A–3–a is to be amended as follows:

"III–A–3–a. *Purified DNA Other than Plasmids, Bacetriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified [41] and in which the absence of harmful sequences has been established [3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment [42]. The containment may be decreased one step in physical containment (P4→P3; P3→P2; P2→P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (HV3→HV2; HV2→HV1) while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1+HV1, which requires prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–(3)–(e).)"

B. Section III–A–3–b is to be amended as follows:

"III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (3) experiments involving this recombinant DNA may be carried out under lower containment conditions.

Institutional biosafety committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–b to levels below P1+HV1, or reduction of containment levels by more than step, which also require prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–3–(e).)"

C. Section IV–D–1–c–(3) is to be amended as follows:

"IV–D–1–c–(3). Certain reductions of containment levels for characterized or purified DNA preparations or clones (see Section III–A–3)."

D. The Note in Section IV–D–3–b is to be amended as follows: (Additional language in Italics).

"Note.—Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other examples are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. *Still others are single-step reductions in containment level for (i) experiments with DNA recombinants from cellular DNAs that have been purified and are judged to be free of harmful sequences (See Section III–A–3–a) and for (ii) clones that have been characterized and judged to be free of harmful sequences (see Section III–A–3–b).* It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion."

E. Section IV–E–1–b–(3)–(e) is to be amended as follows:

"IV–E–1–b–(3)–(e). Lowering containment levels in certain cases for characterized clones or purified DNA (see Sections III–A–3–a and –b, and Footnotes 3 and 41);"

14. *Transfer of DNA Segments Cloned in Saccharomyces cerevisiae Host-Vector Systems to Higher Plants.* Dr. Jane Setlow of Brookhaven National Laboratory, has suggested that the Guidelines include a general provision for cloning plant DNA in *Saccharomyces cerevisiae* and subsequently returning the cloned DNA to the plant. She proposes that the first sentence of Section III–c–7–b be amended to read as follows:

"III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* or *S. cerevisiae* under appropriate containment conditions, may be transferred with the *E. coli* or *S. cerevisiae* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]."

15. *Status of Variola and Whitepox Viruses.* It was suggested at the RAC March 6–7, 1980 meeting that any mention of the variola viruses in the Guidelines be marked with a symbol so that reference could be made to world organization policy regarding these viruses and worldwide efforts at eradication.

Accordingly, it is proposed that Appendix B, Classification of Microorganisms on the Basis of Hazard, be amended so that each mention of Alastrim, Smallpox and Whitepox will be marked with a symbol. A footnote at the bottom of the page bearing this symbol will read:

"All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta)."

16. *Request for Consideration of Appropriate Containment Conditions.* Dr. Paul S. Sypherd of the University of California, Irvine has requested consideration of the containment levels appropriate to the return of *Mucor racemosus* DNA cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus.* In addition, Dr. Sypherd requests permission to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned yeast sequences.

17. *Proposal to Amend Section I–D–6 of the Guidelines.* Dr. Irving Johnson of Lilly Research Laboratories, proposes that Section I–D–6 of the Guidelines be amended, as follows:

A. Delete the second paragraph of Section I–D–6 which currently reads as follows:

"We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale."

B. Amend the third paragraph of Section I–D–6 to read as follows (amended language in italics):

"Experiments in Categories I–D–1 to I–D–6 may be excepted (4) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, NIH, with advice of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(e).) *Those experiments utilizing an E. coli K–12 host-vector system and meeting the containment requirements of Section III–O are excluded from these prohibitions."*

18. *Proposals to Amend Several Sections of Part IV, Roles and Responsibilities.* Dr. Irving Johnson of Lilly Research Laboratories, has proposed several amendments of Part IV of the Guidelines, "Roles and Responsibilities," as follows:

A. Amend Section IV–E–1–b–(3)–(d) under Section IV–E–1–b, *"Specific Responsibilities of the Director, NIH,"* to read as follows (new language in italics):

"IV–E–1–b–(3)–(d). Authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences (see Footnote 3 and Section I–D–6).

Further volume increases at the approved containment level, e.g., for industrial development and production purposes, of previously approved (by the Director of NIH) large-scale experiments may be subsequently approved by the local IBC in accordance with Section IV–D–3–a. The IBC will notify ORDA of such volume increases."

B. Amend the second paragraph of Section IV–E–2, *Recombinant Advisory Committee,* to read as follows (amended language in italics):

"The members of the committee shall be chosen to provide, collectively, expertise in scienctific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics, epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. *Appropriate representatives of industry shall also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production.* At least 20 percent of the members shall be persons knowledgeable in an applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as non-voting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities."

The first and third paragraphs of Section IV–E–2 would remain unchanged.

C. Amend Section IV–E–2, *Recombinant Advisory Committee,* to add a new section IV–E–2–b, as follows:

"IV–E–2–b. A permanent subcommittee of the RAC shall be responsible for advising the Director of NIH, on the actions, listed in Section IV–E–1–b–(3)–(d) pertaining to large-scale applications. Submissions that are in compliance with the Guidelines may be recommended by the subcommittee to the Director of NIH for approval. The subcommittee shall also be authorized to consider preliminary plans for large-scale operations and to recommend approval contingent upon completion of the large-scale facility according to those plans. The subcommittee will be responsible for expeditiously processing applications."

19. *Request for Use of Conjugative Plasmids and Transducing Phages in an Escherichia coli K–12 Host.* Dr. A. I. Bukhari of the Cold Spring Harbor Laboratory has requested permission from the RAC to employ conjugative plasmids or transducing phages in recombinant DNA experiments involving *Escherichia coli* DNA when a small fragment of heterologous DNA is used as a linker. In the specific request, Dr. Bukhari requests approval to use a fragment of Ad_2 DNA as linker DNA.

20. *Request for Exception to a Prohibition to Clone DNA from a Class 3 CDC Agent.* Dr. Sebastiao Baeta Henriques of the Instituto de Ciencias Biologicas, Belo Horizonte, Brazil, requests the RAC to approve an exception to a prohibition to clone genes from *Schistosoma mansoni,* a Class 3 CDC agent, in *E. coli* K–12. This request was considered briefly at the March 6–7, 1980 RAC meeting. Dr. Henriques was requested to provide information as to why he is requesting NIH approval for a non-NIH project to be conducted in Brazil. Dr. Henriques has provided NIH with an additional letter of explanation.

Additional Announcements of the Director, NIH

Section IV–E–1–b–(3)–(d) of the Guidelines gives responsibility to the Director, NIH, for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences." Accordingly, several requests for authorization to culture, on a large scale, recombinant DNA host-vestor systems have been received and reviewed by the NIH.

I. Genentech, Inc.

On the recommendation of the RAC, the following requests from Genentech, Inc. have been approved by the Director, NIH:

A. On April 1, 1980, the Director, NIH approved a request from Genentech, Inc. for the large-scale culture up to 750 liters of EK1 host-vector systems containing a plasmid into which has been ligated a combination of chemically synthesized DNA and cloned cDNA coding for human growth hormone.

B. On April 9, 1980, the Director, NIH approved a request from Genentech, Inc., for the large-scale culture up to 750 liters of EK1 host-vector systems constructed to produce various chimeric fusion proteins containing either somatostatin, human insulin A chain, human insulin B chain, human proinsulin, and thymosin alphal.

Both requests were approved with the understanding that Genetech, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site, and to certain additional stipulations.

The principal investigators are Drs. Michael Ross and Norm S. C. Lin. The work is to be done, as stipulated by the Genentech, Inc. submission, in a "P2 laboratory housing fermentors modified and tested to totally contain the recombinant organisms until they are chemically or physically killed at the end of each fermentation * * * at the research and development facility at 460 Point San Bruno Boulevard, South San Francisco, California 94080."

II. Eli Lilly & Co.

A. On April 7, 1980, the Director, NIH, approved a request from Eli Lilly and Company for large scale culture of an EK1 host-vector system containing a plasmid coding for human proinsulin covalently joined to *E. coli* β-galactosidase, at the P2 and P1–LS levels of containment.

B. On April 7, 1980, the Director, NIH, approved a request to amend a previous submission of Eli Lilly and Company. In the previous submission, which was approved on October 5, 1979 by the Director, NIH, Eli Lilly and Company requested permission to culture on a large-scale EK1 host-vector systems separately carrying the chemically synthesized insulin A chain and B chain. The February 1980 submission requested permission to amend this protocol to permit culture of the same host-vector systems in larger volumes at the P1–LS level of containment.

C. On April 9, 1980, the Director, NIH approved a joint request from Eli Lilly and Company and Genentech, Inc., for large scale culture of EK1 host-vector systems producing human proinsulin, insulin A chain, and insulin B chain as chimeric fusion proteins in RAC-approved fermentors of Eli Lilly and Company at the P2 and P1–LS levels of containment.

These requests were approved with the understanding that Lilly Research Laboratories have agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigator for these projects is Dr. Lawrence E. Day. The large scale growth of the organisms is to be carried out at plant facilities located at 1200 South Kentucky Avenue, Indianapolis, Indiana 46206.

Dated: April 23, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

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